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(57) Abstract

A MORT1 gene initially cloned from HeLa cells and identified as a member of the receptor mediated apoptotic pathway, is expressed in the human neuronal cell line, NTERA2. Isolation of the MORT1 from this cell line revealed a transcript isoform that differed from the known MORT1 sequence by a deletion of 21 base pairs (bp 172–192 of the coding sequence). Cloning of MORT1 from adult human brain revealed two isoforms, one similarly deleted for bp 172–192, the other with a basepair substitution, A for G at position 173. Assessment of MORT1 function in a yeast two hybrid system indicates that the deleted and intact forms of MORT1 differ in their capacity to interact with other members of the apoptotic pathway.

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Neuronal MORT1 Isoforms

Field of the Invention

The present invention relates to human intracellular death-domain-containing apoptosis signaling proteins. In particular, the present invention relates to novel human neuronal MORT1 isoforms.

Background

Apoptosis, or programmed cell death in multicellular organisms, is one of the fundamental means by which a cell can respond to environmental changes. One of the best studied mammalian apoptosis systems involves Fas (also designated APO-1 and CD95), a type I membrane receptor that, when crosslinked by its cognate ligand, induces apoptosis in a wide variety of cells (for review, see Nagata, 1994). The extracellular interaction of Fas ligand with the cell membrane-spanning Fas receptor activates an intracellular signal transduction cascade finally activating proteases in the IL-1β-converting enzyme (ICE) family (Henkart, 1996). Transduction of an apoptosis signal depends on interaction between the intracellular "death domain" of Fas with a cytoplasmic 23-kDa protein, MORT1 [(Boldin, *et al.*, 1995), also termed FADD (Chinnaiyan, *et al.*, 1995)]. The events leading from the production of an activated Fas trimer complex to cell destruction mediated by ICE-like proteases are yet to be determined, but recruitment of two MORT1/FADD molecules into a death-inducing signaling complex with the death domain of Fas appears to be a necessary step (Kischkel, *et al.*, 1995). The end result of this pathway is cell death by a distinctive mechanism characterized by nuclear and cytoplasmic condensation and DNA fragmentation.

The human MORT1/FADD gene spans approximately 3.6 kb and contains two exons (286 and 341 bp) separated by a 2.0-kb intron. MORT1/FADD was mapped to chromosome 11q13.3 by the independent techniques of PCR screening of somatic cell hybrid mapping panels and

fluorescence *in situ* hybridization (Kim, *et al.*, 1996). Knowledge of the chromosomal location and gene structure of MORT1/FADD will assist efforts to determine its involvement in genetic disorders of apoptosis. Defects in apoptosis due to mutations in the Fas receptor have been described (Fisher, *et al.*, 1995; Rieux-Laucat, *et al.*, 1995) in patients with a rare autoimmune lymphoproliferative syndrome (ALPS) including nonmalignant lymphadenopathy, hepatosplenomegaly, and expanded populations of $CD3^+CD4^-CD8^-$ lymphocytes. However, the existence of some patients with clinical findings of ALPS, but no Fas mutation, suggests that defects in other proteins in the Fas pathway may also produce ALPS. The functional involvement of MORT1/FADD in the Fas pathway makes it a candidate for mutation analysis in ALPS.

Localization of MORT1/FADD to 11q13.3 also makes it a candidate for human diseases associated with this part of the genome. IDDM4, a predisposing locus for familial insulin-dependent diabetes mellitus (IDDM) has been mapped to this region by linkage studies (Cordell, *et al.*, 1995; Davies, *et al.*, 1994; Hashimoto, *et al.*, 1995). The pathogenesis of IDDM may involve autoimmune T lymphocyte-mediated destruction of pancreatic β islet cells that produce insulin (Tisch and McDevitt, 1996). MORT1/FADD's chromosomal location, coupled with its known role in lymphocyte apoptosis, makes it a candidate for mutational analysis in patients with IDDM4-linked familial diabetes.

In addition, the 11q13 region is amplified in several human malignancies, including carcinoma of the breast, bladder, esophagus, head and neck, and lung (Schuuring, 1995; Szepetowski, *et al.*, 1995). Amplification of this region has been associated with poor prognosis in patients with operable breast cancer (Schuuring, *et al.*, 1992). The mapping of MORT1/FADD to the amplicon of the breast cancer cell line MDA-MB-134-VI (Lafage, *et al.*, 1992) raises the possibility of its involvement in tumor growth. Future linkage and mutation analysis of MORT1/FADD in other diseases may support the hypothesis that

disregulation of cell death is a fundamental mechanism for the pathogenesis of human disease (Thompson, 1995).

A single gene can encode more than one mRNA transcript by transcriptional processing events such as use of alternative promoters, alternative splicing, and alternative polyadenylation (Farrow, 1997; Lewin, 1994). The resulting variant transcript isoforms may differ in stability, translatability, or protein sequence encoded, each of which may impact upon the function of the encoded protein. Transcript variants resulting from alternative RNA processing can be tissue-specific, developmentally regulated, endocrine regulated, or can appear in response to specific exogenous cues. In addition, transcript variants of a gene can result from differences in genomic sequence (among individuals or among cell lines). To date, only one transcript isoform has been reported for the MORT1/FADD gene (Boldin, et al., 1995; Chinnaiyan, et al., 1995). This invention describes the identification of two new isoforms of MORT1.

Summary of the Invention

The present invention is based on the discovery of novel transcript isoforms of the human neuronal MORT1gene. The transcript isoforms and the proteins encoded by them are useful as screening agents in the diagnosis of CNS diseases and in the discovery of CNS-specific anti-apoptotic compounds. The DNA, RNA, and proteins encoded by them are useful in a "gene therapy" scenario, either as *in vivo* therapeutic agents in humans, or as experimental tools in manipulating neuronal apoptosis in cell culture and animal model systems.

Accordingly, in one embodiment, the invention is directed to a neuronal protein encoded by an MORT1 gene, wherein the gene is isolated from NTERA2 or adult human brain tissue. The cDNA encoding the neuronal protein differs from the known MORT1 gene by the deletion of 21 base pairs and by base pair substitutions.

Decription of the Figures

Figure 1: Investigation of the MORT1 transcript isoforms with MACHα1 C360S in a yeast two-hybrid system: Activation of the HIS3 reporter gene. Each MORT1 transcript isoforms was expressed as a fusion protein with Gal4 DNA binding domain. MACHα1 C360S was expressed as a fusion protein with the Gal4 activation domain. Yeast strains were plated at a cell density of 5×10^1, 5×10^2, 5×10^3, 5×10^4 and 5×10^5 (left to right within a plate. Sibling isolates from yeast strains expressing a MORT1 transcript isoform with MACHα1 C360S fusion proteins were plated on histidine deficient media containing 0, 20 or 40 mM 3-aminotriazole (3-AT). The top row of plates (1) contained yeast strains that express two fusion proteins as follows: YCB5: MORT1-pAS1 / MACHα1 C360S -pACTII; YCB9: MORT1Δ21-pAS1 / MACHα1 C360S -pACTII; YCB16: MORT1 G173A -pAS1 / MACHα1 C360S -pACTII. The bottom row of plates (3) contains negative control strains which express only the MORT1 transcript isoform fusion protein with a recombinant vector containing nonrelated heterologous DNA, as follows: YCB5.1: MORT1 -pAS1 / SNF4 -pACTII; YCB9.1: MORT1 G173A / SNF4-pACTII; YCB16.1: MORT1Δ21 -pAS1 / SNF4-pACTII. Functional interaction of the MORT1 transcript isoform fusion protein with the MACHα1C360S fusion protein reconstitutes the function of the Gal4 protein and drives HIS3 reporter gene activity thereby linking MORT1 isoform / MACHα1C360S interaction to histidine prototrophy and yeast cell growth.

Figure 2: Investigation of the MORT1 transcript isoforms with MACHα1 C360S in a yeast two-hybrid system: Activation of the CYH2 reporter gene. Each MORT1 transcript isoforms was expressed as a fusion protein with Gal4 DNA binding domain. MACHα1 C360S was expressed as a fusion protein with the Gal4 activation domain. Yeast strains were plated at a cell density of 5×10^1, 5×10^2, 5×10^3, 5×10^4 and 5×10^5 (left to right within a plate. Sibling isolates from yeast strains

expressing a MORT1 transcript isoform with MACHα1 C360S fusion proteins were plated on selective media containing 0, 8 or 12 ug cycloheximide / mL media. The top row of plates (1) contained yeast strains that express two fusion proteins as follows: YCB5: MORT1-pAS1 / MACHα1 C360S -pACTII; YCB9: MORT1Δ21-pAS1 / MACHα1 C360S -pACTII; YCB16: MORT1 G173A -pAS1 / MACHα1 C360S -pACTII. The bottom row of plates (3) contains negative control strains which express only the MORT1 transcript isoform fusion protein with a recombinant vector containing nonrelated heterologous DNA, as follows: YCB5.1: MORT1 -pAS1 / SNF4 -pACTII; YCB9.1: MORT1 G173A / SNF4-pACTII; YCB16.1: MORT1Δ21 -pAS1 / SNF4-pACTII. Functional interaction of the MORT1 transcript isoform fusion protein with the MACHα1C360S fusion protein reconstitutes the function of the Gal4 protein and drives CYH2 reporter gene activity thereby linking MORT1 isoform / MACHα1C360S interaction to sensitivity to cycloheximide and abrogation of yeast cell growth.

Detailed Description of the Invention

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, recombinant DNA technology, and yeast biology and genetics, which are within the skill of the art. Such techniques are explained fully in the literature (e. g., Ausubel, *et al.*, 1993; Coico, 1994; Freshney, 1987; Glover, 1985; Griffin and Griffin, 1994; Hanes and Higgins, 1984; Perbal, 1988; Rose *et al.*, 1990; Sambrook, *et al.*, 1989).

All patents, patent applications and publications cited herein, whether supra or infra, are hereby incorporated by reference in their entirety.

In describing the present invention, the following terms will be employed, and are intended to be defined as indicated below. By "MORT1 transcript isoform" is meant a nucleic acid molecule, including DNA, RNA, mRNA, cDNA derived from the mRNA, or even synthetic

DNA, which is derived either directly or indirectly from a MORT1 genomic sequence. As used herein, the term specifically excludes the transcript described in Chinnaiyan, et al. (1995), Boldin, et al. (1995), and Genbank deposit x84709, and as Genbank x84709 MORT1. The term MORT1Δ21 denotes a cDNA isoform isolated from NTERA2 cells (SEQ. ID NO. 1) or adult human brain (SEQ. ID NO. 3) that has a specific 21 base pair deletion (bp 172-192 of the coding sequence) as compared to the published MORT1. The term MORT1G173A (SEQ. ID NO. 5) denotes a cDNA isoform isolated from human brain that has a nucleotide substitution (A for G) at bp position 173 of the MORT1 coding sequence. Herein the A of the MORT1 translational initiation codon is designated position 1, and corresponds to bp 145 of Genbank sequence x84709.

The above terms (MORT1 transcript isoform, MORT1Δ21, MORT1G173A) encompass the genomic MORT1 sequence encoding them, including introns and exons. Alternatively, a transcript isoform can include transcripts of the genomic sequences which lack one or more introns or exons, or transcripts which incorporate noncoding or coding sequences different from those found in the full-length, wild-type genomic sequence. Transcript isoforms may arise by any of a number of naturally occurring processes, including but not limited to mutation, alternative initiation, alternative splicing, and alternative polyadenylation, each of which may affect the primary structure or some other aspect of the function of the encoded protein. The protein product of a transcript isoform may contain amino acids which differ from the wild-type protein due to insertion, deletion, or frameshifting of coding nucleotides. For example, the NTERA2 and the adult human brain isoforms of MORT1 are depicted in SEQ. ID NOS. 1 and 3.

The cDNA encoding intact MORT1 was generated using standard PCR techniques (Finney, 1993; Griffin and Griffin, 1994). HeLa cell cDNA prepared by standard reverse transcriptase/PCR was the source of DNA template. Additionally, MORT1 cDNA was generated using a human

placental cDNA library as the template source. In each case, a MORT1 fragment was amplified by PCR, cloned and sequenced. DNA sequences obtained from placental and HeLa tissue were compared with the MORT1 sequence in Genbank deposit x84709.

The existence of a human neuronal MORT1 transcript was determined by RT/PCR. Human neuronal NTERA2 cells were grown under conditions to promote terminal differentiation to the neuronal phenotype and induced to undergo apoptosis by incubation with staurosporine. RNA extracted from these cells was subjected to RT/PCR using MORT1-specific primers. The resulting MORT1 fragment was cloned and its sequence compared to the MORT1 sequence in Genbank deposit x84709.

MORT1 was also cloned from fetal and adult human brain cDNA libraries by PCR. MORT1 fragments were amplified, cloned, and their sequence compared to the MORT1 sequence in Genbank deposit x84709. In summary, the MORT1 clones deriving from HeLa cells, the human placental library, and fetal human brain matched the Genbank MORT1 sequence. MORT1 clones from NTERA2 cells and three of five clones derived from human adult brain were deleted for bp 172-192 of the MORT1 coding sequence. The other two of the five clones derived from human adult brain had a single base pair substitution (G173A) relative to the Genbank MORT1 sequence. The 21 bp deletion and the G173A substitution both generate a Glu-Pro-Glu amino acid sequence at positions 56-58 of the protein sequence, versus the corresponding Glu-Pro-Gly sequence of the wild-type protein. In addition, the human neuronal MORT1 isoforms include some other basepair substitutions relative to Genbank sequence, as depicted in SEQ. ID NO. 1 and 3.

Human neuronal MORT1 transcript isoforms are also cloned from human cDNA phage libraries by probing with a radiolabelled MORT1 probe using published DNA hybridization methods (Ausubel *et al.*, 1993; Sambrook, et al., 1989). Many human brain sub-region libraries are commercially available (Clontech; Stratagene) for screening. For tissues

or neuronal cell lines for which no commercially available library exists, custom synthesis of a cDNA library is performed from poly(A) + RNA (service available from Clontech). Total RNA and poly(A) selection are performed according to published methods (Glover, 1985; Sambrook, *et al.*, 1989).

Human MORT1 genomic sequence is cloned using standard PCR techniques (Griffin and Griffin, 1994) from human genomic DNA prepared by standard methods (Glover, 1985; Sambrook, et al., 1989).

The relative abundance of neuronal MORT1 transcript isoforms within a given tissue or cell line is assessed by RNase protection and S1 nuclease mapping, performed according to published methods (Ausubel, *et al.*, 1993; Berk and Sharp, 1977; Lee and Costlow, 1987). Specifically, oligonucleotide probes are designed to distinguish the 21 nucleotide difference between the deleted and intact MORT1 isoforms.

A yeast two-hybrid system as described in Young and Ozenberger, WO 95/34646 published December 21, 1995, the whole of which is incorporated herein, was used to functionally characterize the ability of the MORT1 transcript isoforms to interact with MACHα1 C360S, a protein component of the cytoplasmic apparatus of the Fas / APO1 and TNF receptors. Expression vectors were constructed by fusing the GAL4 DNA-binding domain to the MORT1 transcript isoforms. A second expression vector was constructed by fusing the GAL4 Activation domain to MACHα1 C360S. To determine the ability of the MORT1 transcript isoforms to interact with MACHα1 C360S, yeast strains were generated expressing a single MORT1 transcript isoform with MACHα1 C360S, or a single recombinant plasmid encoding a fusion protein with its companion vector containing unrelated heterologous DNA. Strains were tested for productive protein-protein interaction via reporter gene activity and a change in the yeast cell phenotype.

These data suggest that the MORTΔ21 isoform fusion protein is impaired in its ability to functionally interact with MACHα1 C360S fusion

protein. The yeast strains expressing MORTΔ21 with MACHα1 C360S demonstrated decreased histidine prototrophy, and decreased cell growth, as well as decreased sensitivity to cycloheximide and increased cell growth. These were observed in comparison to the growth characteristics of yeast strain expressing the fusion proteins for MORT1 with MACHα1 C360S, or MORTG173A with MACHα1 C360S.

The subject nucleic acids find a wide variety of applications including use as translatable transcripts, hybridization probes, PCR primers, therapeutic nucleic acids, etc.; use in detecting the presence of genes and gene transcripts involved in apoptosis, in detecting or amplifying nucleic acids encoding additional MORT1 homologs and structural analogs, and in gene therapy applications. The gene products encoded by these transcripts have utility in serving as target proteins in the development of therapeutics for the manipulation of the apoptotic pathway.

The following experiments and examples are offered by way of illustration and not by way of limitation.

Examples

Example 1: Cloning of MORT1 from HeLa cells and from a human placental cDNA library.

As a control, the intact MORT1 gene was cloned as described in Boldin, *et al.*, (1995). Oligonucleotides were prepared on an ABI oligosynthesizer, designed according to the published cDNA sequence of human MORT1 (Genbank x84709). A 25 base 5' sense oligonucleotide containing a NcoI site and the start codon ("5' MORT1," 5'-ACC CCG CCA TGG ACC CGT TCC TGG T-3', corresponding to bases -8 to +17), and a 24 base 3' antisense oligonucleotide spanning the stop codon ("3' MORT1," 5'-ACG GGC CCA TCA GGA CGC TTC GGA-3', complementary to bases 636 to 613 of the MORT1 coding sequence), were synthesized. The cDNA encoding intact MORT1 was generated using standard PCR techniques (Finney,1993). Thermal cycling of these reactions was

performed under the regime, 1 min at 95C, then 30 cycles (40 sec at 95C, 1 min at 60C, 1 min at 72C), and 10 min at 72C, using the oligonucleotides "5' MORT1" and "3' MORT1." HeLa cell cDNA and a human placental (matchmaker) cDNA library (Clontech) served as the DNA templates. In each case, a 640 bp fragment was obtained by PCR and subsequently ligated into the cloning vector pCR II (Invitrogen). The HeLa and placental MORT1 cDNA sequences matched the coding sequence of the published MORT1 sequence (Genbank deposit x84709).

Example 2: Cloning of a neuronal MORT1 from NTERA2 cells by RT/PCR using a gene-specific RT primer

In order to investigate whether MORT1 was present in neuronal cells, NTERA-2 cells, which originate from a human teratocarcinoma and represent a late embryonic human neuronal phenotype, were investigated for the presence of MORT1. NTERA2 cells (Stratagene) were grown under conditions to promote terminal differentiation to the neuronal phenotype, including long-term incubation in retinoic acid (Andrews, 1984). Eighteen hours prior to RNA extraction, staurosporine (Calbiochem) was added to the cell culture medium to a final concentration of 100 nM to induce apoptosis. Total RNA extraction was by the RNeasy Total RNA Kit (Life Technologies) using reagents and instructions provided by the manufacturer. Reverse transcription of total RNA was performed with the reagents and instructions for gene-specific reverse transcription provided in the BRL Preamplification Kit (Life Technologies). Specifically, a 27 base 3' antisense oligonucleotide ("MORT1 downstream," 5'-TAG ATG CCT GTG GTC CAC CAG CGC AAA-3', complementary to bases 663 to 637 of the MORT1 coding sequence) was synthesized on an ABI oligosynthesizer. Reverse transcription of 1 µg total RNA was primed using this oligonucleotide.

PCR amplification of NTERA2 cDNA was performed using DNA Taq polymerase and other reagents provided by the manufacturer (Life Technologies) and equimolar amounts of oligonucleotides "5' MORT1"

and "3' MORT1" (see above). Thermal cycling of the PCRs was in the Gene Amp 2400 thermal cycler (Perkin Elmer) under the regime, 1 min at 95C, 35 cycles (15 sec at 95C, 30 sec at 50C, 1 min at 72C), 7 min at 72C. The resulting PCR product (the cDNA corresponding to the MORT1Δ21 isoform) was agarose gel purified using Qiaex II reagents and instructions provided by the manufacturer (Qiagen) and ligated to the pCR2.1 cloning vector (Invitrogen). Recombinant plasmids were obtained by standard methodology, including transformation into One Shot *E. coli* cells (Invitrogen), growth of transformed bacterial cells on LB-agar plates containing 100 µg/mL ampicillin (Sigma), growth of bacterial colonies in LB Medium (Life Technologies,), and preparation of plasmid DNA using the QIAprep Spin Miniprep Kit (Qiagen). DNA sequence was obtained using cycle sequencing dideoxy terminator reagents (ABI) and synthetic oligonucleotides (see below) reacted in a Gene Amp 9600 thermal cycler (Perkin Elmer) under manufacturer's instructions. Reactions were run and data were generated on an ABI automated sequencer. Sequence data were analyzed using Lasergene software (DNA Star).

Oligonucleotides for use in DNA sequencing were prepared (on an ABI synthesizer), designed according to the sequence of the polylinker region of pCR2.1 and published MORT1 sequence. The following oligonucleotides were generated: "TA Forward" (5'-CAG GAA ACA GCT ATG ACC ATG-3', corresponding to the sense strand of the lacZ gene in pCR2.1, 67 bp upstream of the TA cloning site), "TA Reverse" (5'-ACG TTG TAA AAC GAC GGC CAG-3', corresponding to the antisense strand of the lacZ gene in pCR2.1, 112 bases downstream of the TA cloning site), "MORT 150" (5'-ACC TCT TCT CCA TGC TGC TG-3', sense strand, corresponding to bases 131 to 150 of the MORT1 coding sequence), "MORT 230" (5'-TCG AAG TCG TCG ACG CGC CG-3', antisense strand, complementary to bases 248 to 229 of the MORT1 coding sequence), "MORT 400" (5'-TCG ACA GCA TCG AGG ACA GA-3', sense strand, corresponding to bases 377 to 396 of the MORT1 coding sequence), and

"MORT 420" (5'-GAT TCT CAG TGA CTC CCG CA-3', antisense strand, complementary to bases 441 to 422 of the MORT1 coding sequence).

Example 3: **Cloning of MORT1 from NTERA2 cells using nested primers**

NTERA2 cells (Stratagene) were grown under conditions to promote terminal differentiation to the neuronal phenotype, including long-term incubation in retinoic acid (Andrews, 1984). Eighteen hours prior to RNA extraction, staurosporine (Calbiochem) was added to the cell culture medium to a final concentration of 100 nM to induce apoptosis. Total RNA extraction was by the BRL RNeasy Total RNA Kit (Life Technologies, Inc.) using reagents and instructions provided by the manufacturer. Reverse transcription of total RNA was performed with the reagents and instructions provided in the BRL Preamplification Kit (Life Technologies, Inc.). Specifically, reverse transcription of 4 µg total RNA was primed using oligo(dT).

Oligonucleotides for use in PCR were prepared (on an ABI oligosynthesizer), designed according to the published MORT1 sequence (Genbank x84709). A 25 base 5' sense oligonucleotide ("MORT1 upstream," 5'-AAG CGG CGA GAC CTG GCC ACG GCC A-3', corresponding to bases -90 to -66, relative to the translational start site) was synthesized. PCR amplification of NTERA2 cDNA was performed using DNA Taq polymerase and other reagents provided by the manufacturer (Life Technologies) and equimolar amounts of "MORT1 upstream" and "MORT1 downstream" (see above) oligonucleotides. Thermal cycling of the PCRs was in the Gene Amp 2400 thermal cycler (Perkin Elmer, Inc.) under the regime, 1 min at 95C, 35 cycles (15 sec at 95C, 30 sec at 50C, 1 min at 72C), 7 min at 72C. A 2 µL aliquot of the 100 µL reaction served as the DNA template for further PCR amplification using equimolar amounts of oligonucleotides "5' MORT1" and "3' MORT1" (see above) under the same thermal cycling regime.

The resulting PCR product (the cDNA corresponding to the MORT1Δ21 isoform) was agarose gel purified using Qiaex II reagents and

instructions provided by the manufacturer (Qiagen) and ligated into the pCR2.1 cloning vector (Invitrogen). Recombinant plasmids were obtained by standard methodology, including transformation into One Shot *E. coli* cells (Invitrogen), growth of transformed bacterial cells on LB-agar plates containing 100 µg/mL ampicillin (Sigma), growth of bacterial colonies in LB Medium (Life Technologies), and preparation of plasmid DNA using the QIAprep Spin Miniprep Kit (Qiagen). DNA sequence was obtained using cycle sequencing dideoxy terminator reagents (ABI) and synthetic oligonucleotides (see above) reacted in a Gene Amp 9600 thermal cycler (Perkin Elmer) under manufacturer's instructions. Reactions were run and data were generated on an ABI automated sequencer. Sequence data were analyzed using Lasergene software (DNA Star).

Example 4: **Cloning of MORT1 isoforms from brain tissue**

The MORT1 cDNA from human fetal brain was generated using standard PCR techniques. Thermal cycling was performed, using the regime, 1 min at 95C, then 30 cycles (40 sec at 95C, 1 min at 60C, 1 min at 72C), 10 min at 72C, and using the oligonucleotides "5' MORT1" and "3' MORT1" (see above). A human fetal brain (matchmaker) cDNA library (Clontech) and a human adult whole brain (matchmaker) cDNA library (Clontech) were used as DNA templates for PCR. The PCR products of approximately 640 bp were obtained, ligated to the vector pCR II (Invitrogen), and recombinant plasmids were obtained by standard methodology, including transformation into competent One shot *E. coli* cells, growth of transformed bacterial cells on LB-agar plates containing 100 µg/mL ampicillin (Sigma), growth of bacterial colonies in LB Medium (Life Technologies), and preparation of plasmid DNA using by Wizard Plus Minipreps (Promega) or by Qiagen Midiprep (Qiagen). Recombinant plasmids were confirmed by restriction enzyme digestion. The cDNA sequence was determined by dye-deoxy terminator reaction using reagents and protocols from ABI-Perkin-Elmer and the ABI 373A automated sequencer.

The sequence of each of five clones isolated from human fetal brain cDNA matched the published MORT1 sequence. Five clones isolated from human adult brain cDNA were also sequenced. Three of five clones encoded the isoform that contained the deletion of bp 172-192 of the MORT1 coding sequence; the other two clones encoded a single base pair substitution (G173A) relative to the Genbank (X84709) MORT1 sequence.

Example 5: Cloning of MACHa1 from NTERA2 cell cDNA

NTERA2 cells (Stratagene) were grown under conditions to promote terminal differentiation to the neuronal phenotype, including long-term incubation in retinoic acid (Andrews, 1984). Eighteen hours prior to RNA extraction, staurosporine (Calbiochem) was added to the cell culture medium to a final concentration of 100 nM to induce apoptosis. Total RNA extraction was by the BRL RNeasy Total RNA Kit (Life Technologies, Inc.) using reagents and instructions provided by the manufacturer. Reverse transcription of total RNA was performed with the reagents and instructions provided in the BRL Preamplification Kit (Life Technologies, Inc.). Specifically, reverse transcription of 4 μg total RNA was primed using oligo(dT).

Oligonucleotides for use in PCR were prepared (on an ABI oligosynthesizer), designed according to the published MACH sequence (Boldin, *et al.*, 1996). A 24 base molecule (MACH 5' α, 5'-TTT-AAA-AAG-ATG-GAC-TTC-AGC-AGA-3', spanning the translational start codon of MACH α1 and other MACH isoforms) was synthesized. Another 24 base molecule (MACH 3' α, 5'-ATA-GCA-CCA-TCA-ATC-AGA-AGG-GAA-3', complementary to coding sequence, and spanning the stop codon of MACH α1 and MACH α2. PCR amplification of NTERA2 cDNA was performed using DNA Taq polymerase and other reagents provided by the manufacturer (Life Technologies) and equimolar amounts of oligonucleotides MACH 5' α and MACH 3' α (see above). Thermal cycling of the PCRs was in the Gene Amp 2400 thermal cycler (Perkin

Elmer) under the regime, 1 min at 95C, 35 cycles (15 sec at 95C, 30 sec at 50C, 1 min at 72C), 7 min at 72C. Two PCR products, both between 1.4 and 1.5 kb resulted. The larger product (the cDNA corresponding to the MACH α1) was agarose gel purified using Qiaex II reagents and instructions provided by the manufacturer (Qiagen) and ligated to the pCR2.1 cloning vector (Invitrogen). Recombinant plasmids were obtained by standard methodology, including transformation into One Shot *E. coli* cells (Invitrogen), growth of transformed bacterial cells on LB-agar plates containing 100 μg/mL ampicillin (Sigma), growth of bacterial colonies in LB Medium (Life Technologies,), and preparation of plasmid DNA using the QIAprep Spin Miniprep Kit (Qiagen). DNA sequence was obtained using cycle sequencing dideoxy terminator reagents (ABI) and synthetic oligonucleotides reacted in a Gene Amp 9600 thermal cycler (Perkin Elmer) under manufacturer's instructions. Reactions were run and data were generated on an ABI automated sequencer. Sequence data were analyzed using Lasergene software (DNA Star).

Example 6: Interaction of MORT1 transcript isoforms with MACHa1 C360S

To investigate the interaction of MORT1 transcript isoforms, a two-hybrid interaction paradigm was first established with MORT1 and MACHa1 C360S. Genes encoding the fusion proteins were generated by cloning MORT1 and MACHa1 C360S cDNA sequences into plasmids containing the coding regions for the domains of the yeast transcriptional activator proteins, Gal4. DNA binding domain (Gal4) fusion are constructed in pAS1 having a 2m origin of replication which is described in Wade-Harper *et al.*, (1993). Gene activation domain (Gal4) fusions are constructed in pACTII (described in Durfee *et al.*, 1993). The yeast system used in this study is as described by Young and Ozenberger (1995).

The MORT1 cDNA is obtained as a NcoI - Bam HI fragment from MORT1-pCRII plasmid and directionally cloned into pAS1 via NcoI and BamHI restrictions sites to generate MORT1-pAS1 plasmid. The MACHa1

wildtype protein is lethal in yeast. Mutation of the cysteine at amino acid position 360 to a serine prevents the lethal phenotype and does not interfere with the protein's ability to interact with MORT1 (Boldin, *et al.*, 1996). The amino acid substitution to generate MACHa1 C360S was obtained using standard PCR techniques (Finney,1993). Thermal cycling of these reactions was performed under the regime, 1 min at 95C, then 30 cycles (40 sec at 95C, 1 min at 60C, 1 min at 72C), and 10 min at 72C, and using specific oligonucleotides. All oligonucleotides were prepared on an ABI oligosynthesizer and are designed according to published cDNA for human MACH (Boldin *et al.*, 1996; Genbank X98172-X98178). Four oligonucleotides were prepared. A 36 base 5' mutagenic oligonucleotide (oCB1) contained the C360S substitution (TGT to TCT) and a silent mutations at Ala 359 (GCT to GCA) to encrypt a diagnostic restriction enzyme site for SfiI [5'-GTG TTT TTT ATT CAG GCA TCT CAG GGG GAT AAC TAC - 3']. A 36 base 3' mutagenic oligonucleotide (oCB2) [5'-GTA GTT ATC CCC CTG AGA TGC CTG AAT AAA AAA CAC-3'] contained sequence complimentary to oCB1. A 34 base oligonucleotide (oCB3) containing a BamHI site [5'-CGG GAT CCG TAT GGA CTT CAG CAG AAA TCT TTA T-3']. A 38 base 3' oligonucleotide (oCB4) contained a BamHI and SalI restriction enzyme sites [5'-CGG GAT CCG ACG TCG ACT CAA TCA GAA GGG AAG ACA AG-3']. The MACHa1-pCR2.1 plasmid (see example 5, above) was used as template with oligonucleotide pair oCB2 and oCB3 to generate a 1100 basepair fragment, and as template for oligonucleotide pair oCB1 and oCB4 to generate a 360 bp fragment. A subsequent PCR reaction was performed using the 1100 and 360 basepair fragments with oligonucleotide pair oCB3 and oCB4 to generate a 1460 bp fragment which was ligated to pCRII vector (Invitrogen) to generate MACHa1 C360S -pCRII. Recombinant plasmids were obtained by standard methodology, including transformation into One Shot *E. coli* cells (Invitrogen), growth of transformed bacterial cells on LB-agar (Gibco, Inc.)

plates containing either 100 mg/mL ampicillin (Sigma) or 50 mg/mL Kanamycin (Sigma) as described in the manufacturer's (Invitrogen) standard protocol. Growth of bacterial colonies was in LB medium and preparation of plasmid DNA was performed using Qiagen Midiprep (Qiagen) or Wizard Plus Minipreps (Promega). Recombinant plasmids were confirmed restriction enzyme digestion. The cDNA sequence was obtained by dye-deoxy terminator reactions using reagents and protocols from ABI-Perkin-Elmer and the ABI 373A automated sequencer. The cDNA encoding MACHa1 C360S was obtained as a BamHI - SalI fragment and directionally cloned into pACTII via BamHI - XhoI sites to generate MACHa1 C360S - pACTII. DNA of final recombinant vectors were transformed into yeast strain(s) by the lithium acetate method (Rose *et al.*, 1990).

A yeast host strain (CY770) as described in Young and Ozenberger (1995) was transformed with both the MORT1 and MACHa1 C360S fusion constructs (YCB5) or a single fusion construct plus the opposing vector containing unrelated heterologous DNA (YCB5.1 or YCB2.1 , respectively). All strains were found to exhibit equal growth on nonselective medium. Strains were then tested for growth on selective medium (*i.e.*, growth medium lacking histidine). Only cells expressing both the MORT1 and MACHa1 C360S fusions are able to grow on selective medium while the strains containing either the MORT1 or the MACHa1 C360S fusion with an unrelated fusion cannot grow (data not shown).

The MORT1 G173A SEQ. (ID NO. 5) was obtained as a NcoI-BamHI fragment from MORT1 G173A-pCRII and directionally cloned into pAS1 to generate MORT1 G173A-pAS1 plasmid. MORT1 Δ21 was obtained as a NcoI-BamHI fragment from MORT1Δ21-pCRII and directionally cloned into pAS1 to generated MORT1Δ21-pAS1 plasmid. These plasmids and the MACHa1 C360S -pACTII plasmid were used to transform the yeast host strain, CY770. Strains were generated that

express MORT1 G173A and MACHa1 C360S fusion constructs (YCB16) or the MORT1 G173A fusion construct plus the opposing vector containing unrelated heterologous DNA (YCB16.1). Strains were generated that express MORT1Δ21 and MACHa1 C360S fusion constructs (YCB9) or the MORT1Δ21 fusion construct plus the opposing vector containing unrelated heterologous DNA (YCB9.1).

Two independent samples of each strain were streaked on standard synthetic medium containing 0, 20 or 40 mM 3-aminotriazole (Figure 2). Plates were incubated at 30C for 3 days. All strains were found to exhibit equal growth on non-selective medium. Strains were then tested for growth on selective medium (*i.e.*, growth medium lacking histidine). Cells expressing both the MORT1 and MACHa1 C360S fusions, and cells expressing the MORT1 G173A isoform and MACHa1 C360S fusions, were able to grow on selective medium while the strains containing the MORT1Δ21 and MACHa1 C360S fusions were not able to grow (Figure 2). Negative control stains expressing any of the MORT1 isoform fusions or the MACHa1 C360S fusion with an unrelated fusion cannot grow.

These data suggest that the MORT1Δ21 isoform fusion protein is impaired in its ability to functionally interact with the MACHa1 C360S fusion protein.

Example 7: Screen for compounds affecting the interaction of MORT1 transcript isoforms with other interacting proteins (MACH family members)

The screening methods as described in Young and Ozenberger, WO 95/34646 published December 21, 1995, the whole of which is incorporated herein, are used to identify compounds that affect the interaction of MORT1 transcript isoforms with proteins such as MACHa1 C360S.

Low copy number plasmids expressing MORT1 transcript isoforms (MORT1 G173A or MORT1Δ21) and MACHa1 C360S as GAL4 fusion

proteins are constructed to reduce expression of these proteins. cDNA inserts encoding the MORT1 transcript isoforms are subcloned directly from the recombinant pAS1 vector(s) to pUN30AS via NcoI-BamHI fragment to generate either MORT1 G173A-pUN30AS or MORT1 D 21-pUN30AS. The MACHa1 C360S is subcloned directly from the MACHa1 C360S - pCR II as a BamHI-Sal1 fragment into pUN100ACT via BamHI - XhoI sties to generate MACHa1 C360S - pUN100ACT. These plasmids are transformed into yeast strain CY770 (Young and Ozenberger, 1995) with reporter plasmid pOZ146 (Young and Ozenberger, 1995) to generate yeast strain YCB6. The presence of all three plasmids is necessary to confer the necessary phenotype to enable a rescue screen method as described in Young and Ozenberger (1995).

The yeast strain (YCB18) containing the MORT1 G173A and MACHa1 C360S fusion plasmids plus the reporter plasmid, or the yeast strain (YCB17) containing the MORTD21 and MACHa1 C360S plus the reporter plasmid, forms the basis of a simple primary screen for compounds that disrupt the interaction of the MORT1 transcript isoform gene products and MACHa1 C360S.

Example 8: Screen for compounds affecting the interaction of MORT1 transcript isoforms and protein containing a death domain motif (TRADD, Fas/APO1 receptor, TNFR)

The screening methods as described in Young and Ozenberger, WO 95/34646 published December 21, 1995, the whole of which is incorporated herein, are used to identify compounds that affect the interaction of MORT1 transcript isoforms with proteins containing a death domain motif.

Low copy number plasmids expressing MORT1 transcript isoforms (MORT1 G173A, or MORT1Δ21) and the cytoplasmic domain of the TNF receptor containing a death domain motif as GAL4 fusion proteins are constructed to reduce expression of these proteins. cDNA inserts encoding the MORT1 transcript isoforms are subcloned directly from the

MORT1 G173A- pAS1 or the MORT1Δ21-pAS1 recombinant to pUN30AS via NcoI-BamHI fragment to generate either MORT1 G173A-pUN30AS or MORT1Δ21-pUN30AS. The cytoplasmic domain of the TNFR is subcloned directly from TNFcyto-pCR II as an EcoRI fragment into pUN100ACT via EcoRI sites to generate TNFRcyto - pUN100ACT. Plasmids are transformed into the yeast strain CY770 (Young and Ozenberger, 1995) with the reporter plasmid pOZ146 (Young and Ozenberger, 1995). The presence of three plasmids is necessary to confer the necessary phenotype to enable a rescue screen method as described in Young and Ozenberger (1995). The yeast strain (YCB21) containing the MORT1 G173A and TNFRcyto fusion plasmids plus the reporter plasmid, or the yeast strain (YCB20) containing the MORT1Δ21 and TNFRcyto plus the reporter plasmid, forms the basis of a simple primary screen for compounds that disrupt the interaction of the MORT1 transcript isoform gene products and a death domain containing protein, TNFR cytoplasmic domain.

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SEQUENCE LISTING

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Bingham, Brendan W
Young, Kathleen H
Birsan, Camelia

(ii) TITLE OF INVENTION: Neuronal MORT1 Isoforms

(iii) NUMBER OF SEQUENCES: 6

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(F) ZIP: 07054

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US
(B) FILING DATE:
(C) CLASSIFICATION:

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(B) REGISTRATION NUMBER: 34,988
(C) REFERENCE/DOCKET NUMBER: AHP-97147

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(B) TELEFAX: (973) 683-4117

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 606 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..606

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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Ile Cys Asp Asn Val Gly Lys Asp Trp Arg Arg Leu Ala Arg Gln Leu
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Lys Val Ser Asp Thr Lys Ile Asp Ser Ile Glu Asp Arg Tyr Pro Arg
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CGT GAC CTC CAG AAC AGG AGT GGG GCC ATG TCC CCG ATG TCA TGG AAC      576
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(2) INFORMATION FOR SEQ ID NO:2:

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(A) LENGTH: 202 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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    50                  55                  60

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Ala Gly Ala Ala Pro Gly Glu Glu Asp Leu Cys Ala Ala Phe Asn Val
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Ile Cys Asp Asn Val Gly Lys Asp Trp Arg Arg Leu Ala Arg Gln Leu
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Arg Asp Leu Gln Asn Arg Ser Gly Ala Met Ser Pro Met Ser Trp Asn
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Ser Asp Ala Ser Thr Ser Glu Ala Ser  *
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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 606 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..606

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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Met Asp Pro Phe Leu Val Leu Leu His Ser Val Ser Ser Ser Leu Ser
  1               5                  10                  15

AGC AGC GAG CTG ACC GAG CTC AAG TTC CTA TGC CTC GGG CGC GTG GGC        96
Ser Ser Glu Leu Thr Glu Leu Lys Phe Leu Cys Leu Gly Arg Val Gly
            20                  25                  30

AAG CGC AAG CTG GAG CGC GTG CAG AGC GGC CTA GAC CTC TTC TCC ATG       144
Lys Arg Lys Leu Glu Arg Val Gln Ser Gly Leu Asp Leu Phe Ser Met
        35                  40                  45

CTG CTG GAG CAG AAC GAC CTG GAG CCC GAG CTG CTC GCC TCC CTG CGG       192
Leu Leu Glu Gln Asn Asp Leu Glu Pro Glu Leu Leu Ala Ser Leu Arg
    50                  55                  60

CGC CAC GAC CTG CTG CGG CGC GTC GAC GAC TTC GAG GCG GGG GCG GCA       240
Arg His Asp Leu Leu Arg Arg Val Asp Asp Phe Glu Ala Gly Ala Ala
65                  70                  75                  80

GCC GGG GCC GCG CCT GGG GAA GAA GAC CTG TGT GCA GCA TTT AAC GTC       288
Ala Gly Ala Ala Pro Gly Glu Glu Asp Leu Cys Ala Ala Phe Asn Val
                85                  90                  95

ATA TGT GAT AAT GTG GGG AAA GAT TGG AGA AGG CTG GCT CGT CAG CTC       336
Ile Cys Asp Asn Val Gly Lys Asp Trp Arg Arg Leu Ala Arg Gln Leu
            100                 105                 110
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AAA GTC TCA GAC ACC AAG ATC GAC AGC ATC GAG GAC AGA TAC CCC CGC        384
Lys Val Ser Asp Thr Lys Ile Asp Ser Ile Glu Asp Arg Tyr Pro Arg
        115                 120                 125

AAC CTG ACA GAG CGT GTG CGG GAG TCA CTG AGA ATC TGG AAG AAC ACA        432
Asn Leu Thr Glu Arg Val Arg Glu Ser Leu Arg Ile Trp Lys Asn Thr
    130                 135                 140

GAG AAG GAG AAC GCA ACA GTG GCC CAC CTG GTG GGG GCT CTC AGG TCC        480
Glu Lys Glu Asn Ala Thr Val Ala His Leu Val Gly Ala Leu Arg Ser
145                 150                 155                 160

TGC CAG ATG AAC CTG GCG GCT GAC CTG GTA CAA GAG GTT CAG CAG GCC        528
Cys Gln Met Asn Leu Ala Ala Asp Leu Val Gln Glu Val Gln Gln Ala
                165                 170                 175

CGT GAC CTC CAG AAC AGG AGT GGG GCC ATG TCC CCG ATG TCA TGG AAC        576
Arg Asp Leu Gln Asn Arg Ser Gly Ala Met Ser Pro Met Ser Trp Asn
            180                 185                 190

TCA GAC GCA TCT ACC TCC GAA GCG TCC TGA                                606
Ser Asp Ala Ser Thr Ser Glu Ala Ser  *
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(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 202 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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Leu Leu Glu Gln Asn Asp Leu Glu Pro Glu Leu Leu Ala Ser Leu Arg
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Arg His Asp Leu Leu Arg Arg Val Asp Asp Phe Glu Ala Gly Ala Ala
65                  70                  75                  80

Ala Gly Ala Ala Pro Gly Glu Glu Asp Leu Cys Ala Ala Phe Asn Val
                85                  90                  95

Ile Cys Asp Asn Val Gly Lys Asp Trp Arg Arg Leu Ala Arg Gln Leu
            100                 105                 110

Lys Val Ser Asp Thr Lys Ile Asp Ser Ile Glu Asp Arg Tyr Pro Arg
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        115                 120                 125

Asn Leu Thr Glu Arg Val Arg Glu Ser Leu Arg Ile Trp Lys Asn Thr
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Glu Lys Glu Asn Ala Thr Val Ala His Leu Val Gly Ala Leu Arg Ser
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Cys Gln Met Asn Leu Ala Ala Asp Leu Val Gln Glu Val Gln Gln Ala
                165                 170                 175

Arg Asp Leu Gln Asn Arg Ser Gly Ala Met Ser Pro Met Ser Trp Asn
            180                 185                 190

Ser Asp Ala Ser Thr Ser Glu Ala Ser  *
        195                 200
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 627 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..627

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
ATG GAC CCG TTC CTG GTG CTG CTG CAC TCG GTG TCG TCC AGC CTG TCG        48
Met Asp Pro Phe Leu Val Leu Leu His Ser Val Ser Ser Ser Leu Ser
  1               5                  10                  15

AGC AGC GAG CTG ACC GAG CTC AAG TTC CTA TGC CTC GGG CGC GTG GGC        96
Ser Ser Glu Leu Thr Glu Leu Lys Phe Leu Cys Leu Gly Arg Val Gly
            20                  25                  30

AAG CGC AAG CTG GAG CGC GTG CAG AGC GGC CTA GAC CTC TTC TCC ATG       144
Lys Arg Lys Leu Glu Arg Val Gln Ser Gly Leu Asp Leu Phe Ser Met
        35                  40                  45

CTG CTG GAG CAG AAC GAC CTG GAG CCC GAG CAC ACC GAG CTC CTG CGC       192
Leu Leu Glu Gln Asn Asp Leu Glu Pro Glu His Thr Glu Leu Leu Arg
    50                  55                  60

GAG CTG CTC GCC TCC CTG CGG CGC CAC GAC CTG CTG CGG CGC GTC GAC       240
Glu Leu Leu Ala Ser Leu Arg Arg His Asp Leu Leu Arg Arg Val Asp
65                  70                  75                  80

GAC TTC GAG GCG GGG GCG GCA GCC GGG GCC GCG CCT GGG GAA GAA GAC       288
Asp Phe Glu Ala Gly Ala Ala Ala Gly Ala Ala Pro Gly Glu Glu Asp
                85                  90                  95
```

```
CTG TGT GCA GCA TTT AAC GTC ATA TGT GAT AAT GTG GGG AAA GAT TGG      336
Leu Cys Ala Ala Phe Asn Val Ile Cys Asp Asn Val Gly Lys Asp Trp
            100                 105                 110

AGA AGG CTG GCT CGT CAG CTC AAA GTC TCA GAC ACC AAG ATC GAC AGC      384
Arg Arg Leu Ala Arg Gln Leu Lys Val Ser Asp Thr Lys Ile Asp Ser
        115                 120                 125

ATC GAG GAC AGA TAC CCC CGC AAC CTG ACA GAG CGT GTG CGG GAG TCA      432
Ile Glu Asp Arg Tyr Pro Arg Asn Leu Thr Glu Arg Val Arg Glu Ser
    130                 135                 140

CTG AGA ATC TGG AAG AAC ACA GAG AAG GAG AAC GCA ACA GTG GCC CAC      480
Leu Arg Ile Trp Lys Asn Thr Glu Lys Glu Asn Ala Thr Val Ala His
145                 150                 155                 160

CTG GTG GGG GCT CTC AGG TCC TGC CAG ATG AAC CTG GCG GCT GAC CTG      528
Leu Val Gly Ala Leu Arg Ser Cys Gln Met Asn Leu Ala Ala Asp Leu
                165                 170                 175

GTA CAA GAG GTT CAG CAG GCC CGT GAC CTC CAG AAC AGG AGT GGG GCC      576
Val Gln Glu Val Gln Gln Ala Arg Asp Leu Gln Asn Arg Ser Gly Ala
            180                 185                 190

ATG TCC CCG ATG TCA TGG AAC TCA GAC GCA TCT ACC TCC GAA GCG TCC      624
Met Ser Pro Met Ser Trp Asn Ser Asp Ala Ser Thr Ser Glu Ala Ser
        195                 200                 205

TGA                                                                 627
*
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 209 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Met Asp Pro Phe Leu Val Leu Leu His Ser Val Ser Ser Ser Leu Ser
  1               5                  10                  15

Ser Ser Glu Leu Thr Glu Leu Lys Phe Leu Cys Leu Gly Arg Val Gly
            20                  25                  30

Lys Arg Lys Leu Glu Arg Val Gln Ser Gly Leu Asp Leu Phe Ser Met
        35                  40                  45

Leu Leu Glu Gln Asn Asp Leu Glu Pro Glu His Thr Glu Leu Leu Arg
    50                  55                  60

Glu Leu Leu Ala Ser Leu Arg Arg His Asp Leu Leu Arg Arg Val Asp
```

65 70 75 80

Asp Phe Glu Ala Gly Ala Ala Ala Gly Ala Ala Pro Gly Glu Glu Asp
85 90 95

Leu Cys Ala Ala Phe Asn Val Ile Cys Asp Asn Val Gly Lys Asp Trp
100 105 110

Arg Arg Leu Ala Arg Gln Leu Lys Val Ser Asp Thr Lys Ile Asp Ser
115 120 125

Ile Glu Asp Arg Tyr Pro Arg Asn Leu Thr Glu Arg Val Arg Glu Ser
130 135 140

Leu Arg Ile Trp Lys Asn Thr Glu Lys Glu Asn Ala Thr Val Ala His
145 150 155 160

Leu Val Gly Ala Leu Arg Ser Cys Gln Met Asn Leu Ala Ala Asp Leu
165 170 175

Val Gln Glu Val Gln Gln Ala Arg Asp Leu Gln Asn Arg Ser Gly Ala
180 185 190

Met Ser Pro Met Ser Trp Asn Ser Asp Ala Ser Thr Ser Glu Ala Ser
195 200 205

*

What is Claimed is:

1. A composition comprising an isolated polynucleotide from NTERA2 cells selected from the group consisting of:

(a) a polynucleotide comprising the MORT1Δ21 nucleotide sequence set forth in SEQ. ID NO. 1, deposited under accession number ATCC 209013;

(b) a polynucleotide comprising the nucleotide sequence encoding a protein that interacts with the death domain of Fas/APO1 or fragments thereof;

(c) a polynucleotide encoding a protein that interacts with MACH α1 or other members of the ICE/Ced3 (Caspase) family of proteins or fragments thereof;

(d) a polynucleotide which is an allelic variant of the polynucleotide of (a)-(c) above; and

(e) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(d).

2. A composition comprising an isolated polynucleotide from human brain selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence set forth as in SEQ. ID NO. 3, deposited under accession number ATCC 209018;

(b) a polynucleotide encoding a protein that interacts with the death domain of Fas/APO1 or fragments thereof;

(c) a polynucleotide encoding a protein that interacts with MACH α1 or other members of the ICE/Ced3 (Caspase) family of proteins or fragments thereof;

(d) a polynucleotide which is an allelic variant of the polynucleotide of (a)-(c) above; and

(e) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(d).

3. A composition comprising an isolated polynucleotide from human brain selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence set forth in SEQ. ID NO. 5, deposited under accession number ATCC 209019;

(b) a polynucleotide encoding a protein that interacts with the death domain of Fas/APO1 or fragments thereof;

(c) a polynucleotide encoding a protein that interacts with MACH α1 or other members of the ICE/Ced3 (Caspase) family of proteins or fragments thereof;

(d) a polynucleotide which is an allelic variant of the polynucleotide of (a)-(c) above; and

(e) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(d).

4. An isolated MORT1 nucleic acid isoform, wherein the isoform is a human NTERA2 neuronal cell or human brain isoform encoding a protein comprising the amino acid sequences selected from the group consisting of SEQ. ID NO. 2, SEQ. ID NO. 4 AND SEQ. ID NO. 5.

5. An isolated MORT1 nucleic acid isoform comprising the amino acid sequence of SEQ. ID NO. 2.

6. An isolated MORT1 nucleic acid isoform comprising the amino acid sequence of SEQ. ID NO. 4.

7. An isolated MORT1 nucleic acid isoform comprising the amino acid sequence of SEQ. ID NO. 6.

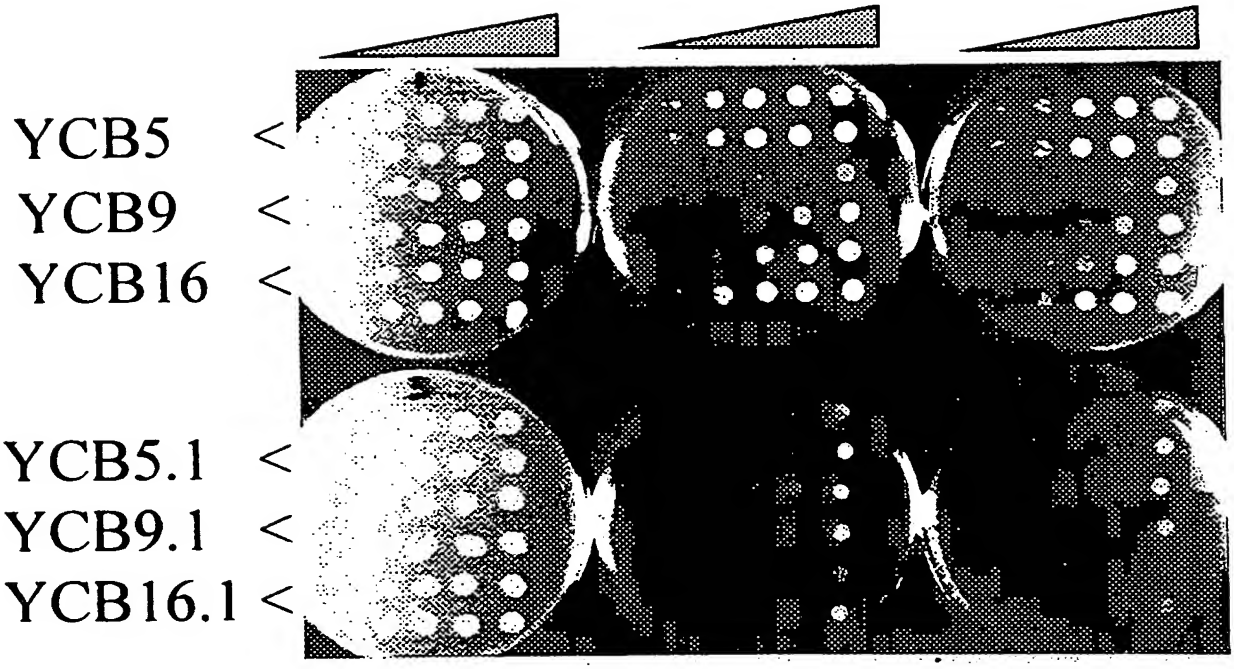

FIG. 1

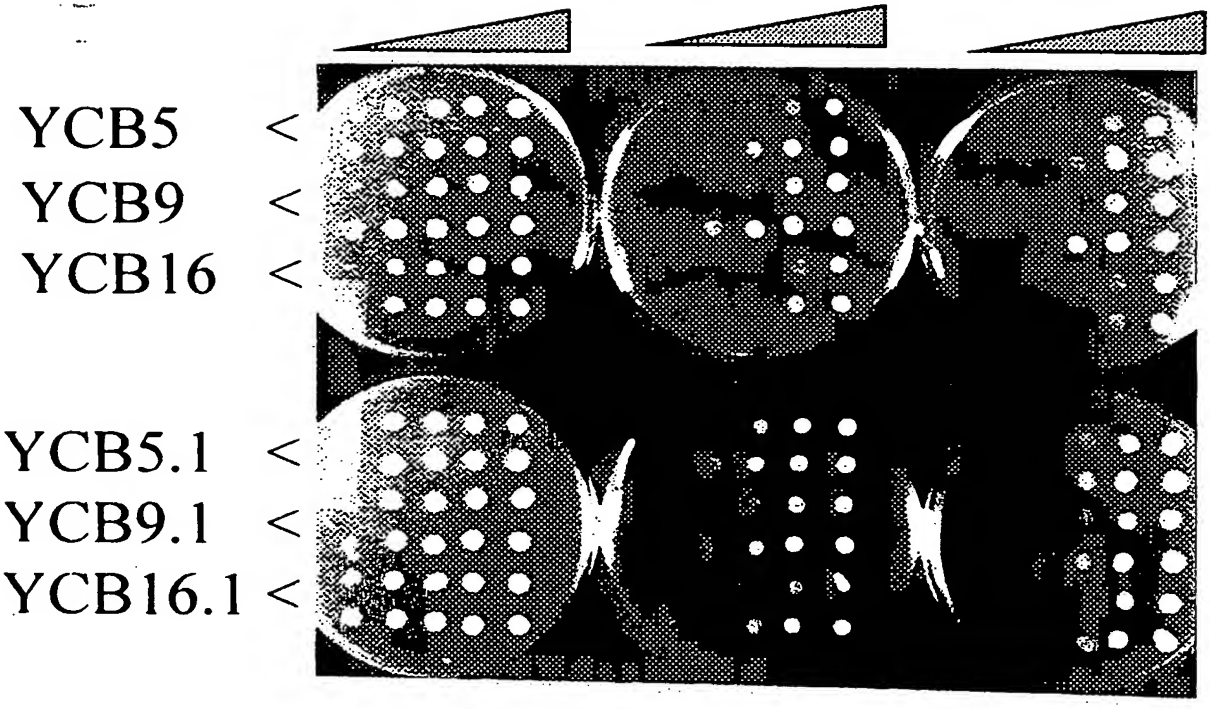

FIG.2

INTERNATIONAL SEARCH REPORT

Interr[illegible]pplication No
PCT/US 98/07439

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C07K14/47 C07K14/715

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 31603 A (UNIV MICHIGAN ;DIXIT VISHVA M (US); ROURKE KAREN O (US)) 10 October 1996 see page 21, paragraph 3; claims 1-14; figure 2; example 1 ---	2
Y	WO 96 18641 A (YEDA RES & DEV ;WEINWURZEL HENRY (IL); WALLACH DAVID (IL); BOLDIN) 20 June 1996 see abstract; claims 1-28; example 1; table 1 --- -/--	1-4

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 September 1998	25/09/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Gurdjian, D

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WANKER, ERICH E. ET AL: "HIP-I: a huntingtin interacting protein isolated by the yeast two-hybrid system" HUM. MOL. GENET. (OCTOBER 25-30 997), 6(3), 487-495 CODEN: HMGEE5;ISSN: 0964-6906, XP002077553 see abstract see page 493, left-hand column, paragraph 2 - page 494, left-hand column, paragraph 2	1-4
X	CHINNAIYAN A M ET AL: "FADD, A NOVEL DEATH DOMAIN-CONTAINING PROTEIN, INTERACTS WITH THE DEATH DOMAIN OF FAS AND INITIATES APOPTOSIS" CELL, vol. 81, no. 4, 19 May 1995, pages 505-512, XP002015439 see the whole document	7
X	WO 96 25941 A (PANCER ZEEV ;GONCHAROV TANYA M (IL); METT IGOR (IL); WALLACH DAVID) 29 August 1996 see claims 1-36; figure SEQ.ID.5	5,6
A	BREDESEN DE: "KEEPING NEURONS ALIVE - THE MOLECULAR CONTROL OF APOPTOSIS.1." NEUROSCIENTIST, 1996, 2, 181-190, XP002077555 see abstract; table 4	1-7
P,X	BINGHAM B ET AL: "Human neuronal sequence variants of the mediator of apoptosis, MORT1" SOCIETY FOR NEUROSCIENCE ABSTRACTS, 23 (1-2). 896.,October 1997, XP002077554 see the whole document	1-4

INTERNATIONAL SEARCH REPORT

Information on patent family members

Interr[illegible]al Application No
PCT/US 98/07439

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9631603 A	10-10-1996	AU 5301296 A	23-10-1996
WO 9618641 A	20-06-1996	AU 4602296 A	03-07-1996
		CA 2207815 A	20-06-1996
		FI 972457 A	10-06-1997
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		AU 6180596 A	18-02-1997
		NO 980198 A	09-03-1998
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WO 9625941 A	29-08-1996	AU 5133296 A	11-09-1996
		CA 2213484 A	29-08-1996
		EP 0813419 A	29-12-1997